Jurnal Biodjati 5(1):138-152, May 2020
e-ISSN : 2541-4208
p-ISSN : 2548-1606
JURNAL BIODJATI
http://journal.uinsgd.ac.id/index.php/biodjati

EFFECTIVENESS OF LACTIC ACID BACTERIA TO IMPROVE *Cyprinus carpio* FINGERLINGS RESISTANCE AGAINST *Edwardsiella tarda* BACTERIAL ATTACK

Rosidah[1]*, Yuniar Mulyani[2], Walim Lili[3], Khasanatur Rosyidah[4]

Selected paper from the 4th Seminar Nasional Biologi (SEMABIO), Bandung-Indonesia, May 18, 2019 (conference.bio.uinsgd.ac.id).
Received : August 23, 2019
Accepted : May 07, 2020

DOI: 10.15575/biodjati.v5i1.5260

[1,2,3,4]Fisheries Studies Program, Faculty of Fisheries and Marine Science, Universitas Padjadjaran, Bandung – Sumedang KM 21, Jatinangor 45363, Indonesia

e-mail:
*[1]ros_ahdi@yahoo.com
[2]yuniarmulyani@gmail.com
[3]walimm78@gmail.com
[4]khasanaturr@gmail.com

**Corresponding author*

Abstract. *Common carp (Cyprinus carpio) cultivation is often hampered by a disease attack, one of them is the attack of Edwarsiella tarda. Lactic acid bacteria (LAB) can be used as an alternative to prevent diseases in fish by increasing the body's resistance. This research aimed to determine the most effective isolates of LAB that increase of the resistance of carp fingerlings to the attack of E. tarda bacteria and see which isolates can produce the highest survival. The LAB isolates used were the result of isolation from the gut of carp. This study used a Completely Randomized Design (CRD) consisting of four treatments with three replications. The fish were immersed with different LAB isolates, CcB7, CcB8 and CcB15 in the same density of 10^8 cells/mm^3. Immersion was carried out for 30 minutes with a frequency of seven days. While during the research, two immersions were carried out before the challenge test against E. tarda bacteria. The parameters observed were the number of leukocytes, hematocrit, erythrocyte, differential leukocytes, survival rate and clinical symptoms that appeared. The results showed that all LAB isolates used in this study could increase the body resistance of carp against the attacks of E. tarda bacteria. The LAB CcB7 isolate was the most effective for enhancing the body resistance of carp fish withthe highest increase level of leukocyte, erythrocyte and hematocrit were 18 ± 0.057; 7 ± 0.077 and 0.26 ± 7.31% respectively. After being challenged with E. tarda bacteria producing mild clinical symptoms, the highest increase is in monocyte and neutrophil cells was 20 and 62% respectively, the highest reduction in lymphocytes was – 9% and the highest survival rate was 80%.*

Keywords: *body resistance, Cyprinus carpio, Edwardsiella tarda, Lactic acid bacteria isolate*

Citation
Rosidah, Mulyani, Y., Lili, W. & Rosyidah, K. (2020). Effectiveness of Lactic Acid Bacteria to Improve *Cyprinus carpio* Fingerlings Resistence Against *Edwardsiella tarda* Bacterial Attack. *Jurnal Biodjati*, 5(1), 138-152.

INTRODUCTION

Common carp (*Cyprinus carpio* L.) is one commodity gains a lot of interest from Indonesian society. The production of carp in West Java in 2016 reached 213,535.97 tons. Many farmers cultivate this fish (Department of Fisheries and Marine, 2018).

Disease attacks often become obstacles in cultivation activities. because it can cause

Jurnal Biodjati 5(1):138-152, May 2020

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

mass death and farmers suffer losses. Since the outbreak of the disease in carp in 2002, farmers are very alert to disease attacks on their cultivation activities (Rahmawati, 2013). One of the bacteria that often causes losses in fish farming activities is *Edwardsiella tarda* (Mohanty & Sahoo, 2008).

E. tarda is a cause of emphysematous Putrefactive Edwardsillosis Disease (EPD). According to Rao et al. (2001), these bacteria cause serious injury to the skin and internal organs such as the liver, kidneys, spleen and muscle. Moreover, these bacteria also attack the body's defense system. The bacterial proliferation process is very rapid within the host leading death.

During this time, fish disease control is conducted using chemicals and antibiotics. Continuous use of antibiotics can cause harmful effects that lead to resistance. The inappropriate dosage use of antibiotics either through feeding, soaking or injection can cause antibiotics accumulation in the body of the fish (WHO, 1998 in Prastiti, 2015). One alternative that can be used as a solution to this problem is prevention by enhancing the immune system of fish.

One way to stimulate non-specific immunity is by using immunostimulant. Probiotic bacteria could be used as an immunostimulant. As opinions of Septriani et al. (2012), the mechanism of action of probiotics is as stimulating the non-specific immune system in fish. Furthermore, the prevention of disease by the use of probiotics is considered more secure than the other methods. One probiotic that can be used is a probiotic containing lactic acid bacteria (LAB).

According to Lopez (2000), in Pinoke et al. (2015), the mechanism of action of LAB bacteria is to reduce the ability of pathogenic microorganisms to live because probiotics can produce antibacterial components such as hydroxy peroxide and organic acids such as lactic acid. As the opinion of Allameh et al. (2017) stated that probiotics could stimulate specific and non-specific immune systems in fish. Because when probiotic bacteria consumed and cover the intestinal mucosal surface, they interact with immune cells in the epithelial layer and the lamina propria of the digestive tract (Bintoro, 2002).

The effectiveness of LAB in increasing body resistance has been proven in several studies. LAB has been shown to increase the non-specific immune response of tiger grouper, by increasing hematocrit, leukocytes, differential leukocytes (lymphocytes, monocytes and neutrophils) and phagocytic activity that are useful in dealing with *Vibrio alginolyticus* (Lestari et al., 2013). Then, in the study of Pimentel & Katagiri (2008), showed that the administration of four species of *Lactobacillus* sp., to tilapia resulted in increasing of macrophage response and lower fish mortality after being challenged with *E. Tarda*.

However, it is important to know which types of lactic acid bacteria that is effective in increasing the body's resistance. Therefore, the aim of this study was to determine the effective isolates of lactic acid bacteria to increase the resistance of carp to the attack of *E. tarda* bacteria and produce the highest survival.

MATERIALS AND METHODS

The research material used including 5-7 cm sized carp Majalaya 120 tail; isolates of lactic acid bacteria, namely CcB7, CcB15 and CcB8 that were isolated from the gut of carp (Rosidah et al. 2017) and *E. tarda* with a density of 10^8 cells/mL.

This experimental method used was completely randomized design (CRD) with 4 treatments and 3 repetitions for each treat-

ment. The treatment used was the provision of lactic acid bacteria (LAB) by immersion with isolates code:
Treatment A: control
Treatment B: isolates CcB7
Treatment C: isolates CcB8
Treatment D: isolates CcB15

Research Procedure

Fish Treatment with LAB

A total of 12 aquariums were used for the experiment. Each aquarium was filled with 5L water, followed by aeration for 24 hours. Each aquarium was then filled with LAB isolate (density of 10^8 cells/mL) according to treatment A, B, C and D. Subsequently, ten carp were added to each aquarium containing LAB for 30 minutes. Each carp had been acclimatized for one week before use for the experiment. The fish were immersed in a LAB solution twice at seven-day intervals (Efendi et al., 2016).

Challenge Test

Challenge test was conducted by injecting *E. tarda* with density of 10^8 cell/mL into the fish body intra-muscularly as much as 0.1 mL/fish. Fish that have been challenged tested were kept for 14 days.

Blood Drawing

Fish blood tests performed three times, before treatment, after treatment and after the challenge test. Blood was drawn from the tail of the fish by cutting the tail of a fish. Blood was taken and then used for observation of red blood cells, white blood cells, hematocrit, and leukocyte differential.

Parameter Observation

Total of Leukocytes After Immersion with LAB Isolates and the Test Challenge with *E. tarda*

Calculation of white blood cells or leukocytes was done by the Klontz (1994) method using a hemacytometer. The blood from the caudal peduncle was taken with a special toma leukocyte pipette of 0.5 to count the number of leukocyte. Then the blood was diluted with hayem's solution until they reach number 11 and homogenized. Furthermore, the number of blood was observed and counted under a microscope with 400x magnification. The cells in the 4 large squares of hemacytometer were counted. The total of leukocytes was determined using the formula:

$$\text{Total leukocytes} = n \times 500 \text{ cells mm}^3$$

n = The number of leukocytes contained in a big box room count
500 = Dilution factor

Total of Erythrocytes After Immersion with LAB Isolates and the Test Challenge with *E. tarda*

Calculation of red blood cells or erythrocytes was based on methods Klontz (1994) using a hemacytometer. A blood sample from the tail rod was taken using a pipette toma with small stones in red to indicate line 0.5 mL. Turks solution was then added until the the overall solution reached 101 mL and homogenized. The sample on hemacytometer was then observed and counted under a microscope with 400x magnification. The cell counting was performed at the small 5 squares in the center of hemocytometer and calculated by the formula:

$$\text{Total erythrocytes} = n \times 10^4 \text{ cells/mm}^3$$

n = The number of erythrocytes contained in the small 5 squares
10^4 = Dilution factor

Total of Hematocrit After Immersion with LAB isolates and the Test Challenge with *E. tarda*

Jurnal Biodjati 5(1):138-152, May 2020

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Measurement of hematocrit in fish was conducted based on Anderson & Siwiciki (1993), by sucking the blood using a heparin-coated capillary tube. The tip of the tube was closed with plasticine. A capillary tube that has been filled with blood was centrifuged at 3500 rpm for 15 minutes. Measurements were made by comparing the volume of blood to the volume of the entire body using a blood hematocrit scale. Hematocrit levels were calculated by the following equation:

$$He = \frac{a}{b} \times 100$$

He = hematocrit level (%)
a = Part of the blood that settles (mm)
b = Parts of whole blood in a tube microhematocrit

Differential of Leukocytes after challenge with *E. tarda*

Leukocyte differential calculation, namely monocytes, neutrophils and lymphocytes, was done by observation of blood pillowcase preparations. The method of making preparations for blood was described by Anderson & Siwicki (1993). The following is the leukocyte differential calculation formula:

$$\% \text{ Cell type} = \frac{number\ of\ cells}{100} \times 100\%$$

Clinical Symptoms of Carp After Infected *E. tarda*

Observation of the carp clinical symptoms started when the fish already infected by the bacteria E. tarda (after the challenge test). The clinical symptoms observed including the onset of injury or damage to the body, in response to the movement and response to a meal. Observations made during the clinical symptoms of 14 days after the challenge test.

The observation of feeding response was carried out directly during the feeding period and examined the food leftover on the bottom of the aquarium.

Survival Rate

Observations of survival rate were done from the first day of carp infected by the bacteria E. tarda until the last day of the maintenance. The percentage of survival was obtained using a formula by Effendie (1997) as follows:

$$SR = \frac{Nt}{No} \times 100\%$$

Information:
SR = Survival rate (%)
Nt = Number of fish alive at end of study
No = Number of fish that live at baseline

Data Analysis

Data on the number of white blood cells (leukocytes), red blood cells (erythrocytes), the measurement of hematocrit and survival rate were analyzed using the F test. In cases there were significant differences among the treatments, the Duncan's multiple range test was used at 5% level (Gaspersz, 1991). Data on clinical symptoms, differential leukocyte and the measurement of hematocrit were analyzed descriptively.

RESULTS AND DISCUSSION

Total of Leukocytes After Immersion with LAB Isolates and the Test Challenge with *E. tarda*

All tested fish that were immersed in LAB isolates (treatment B, C and D) had higher leukocyte count than those not immersed (control). The treatment B, C and D had a greater increase percentage of leukocytes after immersion in LAB than the treatment A, even treatment A decreased number of leukocytes by 13 ± 0.045 %. Even though on the statistical test, the fish that treated with isolates LAB did not provide a real difference, but the treatment B (isolates CcB7) gave the highest increase (18 ± 0.057 %) among all treatments,

while the lowest was in treatment C (isolates CcB8) (2 ± 0.95 %). However, based on the analysis of variance, the percentage number of leukocytes increased significantly in the treatment B, C and D (Table 1). Thus, the immersion of LAB isolates affects the increase in the number of leukocytes.

Immersion the carp fingerlings with LAB has shown to increase the number of leukocytes, meaning that LAB isolates effectively increase their endurance or non-specific immune of the fish. As said Agustina et al. (2006) that increasing the number of leukocytes is one indicator of the increase in the non-specific immune system of the fish's body. Pangaribuan et al. (2013) research results showed the increasing number of fish leukocytes to 77.92 % after the treatment of lactic acid bacteria (bekasang) for 4 weeks compared to fish that were not given the LAB.

Treatment B (CcB7) gave the highest number of leukocytes with the increasing up to 18 ± 0.057 %. This is likely due to the lactic acid isolates produce higher secondary metabolites to release a variety of chemicals that can stimulate cell proliferation and induces immune cells, such as and B lymphocytes, and macrophages. As Bintoro (2002) opinion, the binding process of bacteria and various metabolites lead to the production of organic acids, hydrogen peroxide and bacteriocins that cause the release of a variety of chemotaxis factors, interleukins and interferons. These will stimulate and intensify the process of proliferation of immune cells so that the number is increasing. The opinion was also supported by G'omez & Balc'azar (2008) which stated that the LAB as a natural immunostimulant, especially coming from the digestive tract of fish is a potential candidate to replace antibiotics in controlling diseases in fish. During the growth of lactic acid bacteria, they produce metabolites components such as organic acids, hydrogen peroxide, bacteriocins, other components (Vasiljevic & Shah, 2008). These components will function as an antimicrobial (Amezquita & Brashears, 2002).

The low increase number of leukocytes in the treatment of C (CcB8 bacterial isolates) indicated that these isolates produce low secondary metabolites so that they are unable to induce the proliferation of immune cells that have already existed (natural immunity).

After the challenge test with bacteria *E. tarda* in treatment A, C and D increased the number of leukocytes, where treatment D had the highest count, while on treatment B decreased the number of leukocytes (Table 1).

The carp that has been immersed with LAB isolates increased the number of leukocytes significantly after the challenge test with bacteria E. Tarda, The results of the Duncan test at a 5% level showed the A and C treatment was significantly different from treatment B and D. The increase in the number of leukocytes in treatment B and D was lower compared to other treatments, and even treatment B showed a decreasing percentage of the number of leukocytes by 2 ± 0.057%. While treatment A and C experienced an increasing percentage of the number of leukocytes by 35 ± 0.044 % and 25 ± 0.060% respectively.

An increasing number of leukocytes during the challenge test indicates the fish body's defensive reaction against bacterial pathogens that enter the body (antigen). In accordance with Sukenda et al. (2008) found an increase in total leukocytes in the blood due to faster body defense against the entry of antigen into the body of fish. The declining number of leukocytes in treatment of B infections that occurred after the challenge test may indicate that fish has begun to improve, and fish was in a healthy condition to heal from pathogenic bacterial infections.

Jurnal Biodjati 5(1):138-152, May 2020

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

Table 1. The increase percentage of total leukocytes after immersion with LAB isolates and the Test Challenge with *E. tarda*

Treatment	Total Leukocyte (10^4 cells/mm^3)		The increase persentage of leukocytes (%)	Total Leukocyte (10^4cell/mm^3)		The increase percentage of leukocyte (%)
	Before immersion	After immersion		After immersion	Test challenge (In vivo)	
Control (A)	6.63 ± 0.16	5.60 ± 0.2	-13 ± 0.045^a	5.60 ± 0.2	8.57 ± 0.4	35 ± 0.044^b
CcB7 (B)	8.77 ± 0.28	10.65 ± 0.6	18 ± 0.057^b	10.65 ± 0.6	10.42 ± 0.6	-2 ± 0.057^a
CcB8 (C)	6.47 ± 0.33	6.62 ± 0.6	2 ± 0.95^b	6.62 ± 0.6	8.78 ± 0.2	25 ± 0.060^b
CcB15 (D)	9.08 ± 0.82	10.12 ± 1.1	10 ± 0.026^b	10:12 ± 1.1	10.65 ± 11	5 ± 0.051^a

Description: The means followed by same letter are not significantly different from each other (P>0.05 ANOVA)

Total of Erythrocytes After Immersion with LAB Isolates and the Test Challenge with *E. tarda*

All treatments (A, C and D) decreased the number of erythrocytes after immersion with LAB isolates, except for treatment B that showed the increasing number of erythrocytes. The treatment B generateed the largest number of erythrocytes by 1.13 x 10^6 cells/mm^3 ± 0.03, while the lowest number of erythrocytes found treatment A by 1.01 x 10^6 cells/mm^3 ± 0.02. Based on the analysis of variance, all treatment gave no significant difference in the percentage the number of erythrocytes in the tested fish. However, treatment A, C and D decreased erythrocytes count ranged between 1-3%, whereas treatment B had increased the number of erythrocytes by 7 ± 0.077 % after immersion with LAB isolates. The largest decrease occurred in treatment A by 3 ± 0.047 % (Table 2).

Total erythrocytes fish generally range 1.5-3 x 10^6 cells/mm^3 (Roberts 2001) and the increase in the number of erythrocytes occurs due to the presence of compounds that stimulate an increase of the immune system. Nurjannah et al. (2013) reported that an increase in erythrocytes occurs because of a compound which serves to increase their immunity. In this research, the fish in treatment B showed high increase levels of leukocyte (0.057 ± 18%) after immersion with LAB isolates CcB7, where leukocytes is an indicator of body resistance. however, the declining in treatment A, C and D presumably because the fish are still in a period of adaptation to the treatment of lactic acid bacteria. The number of erythrocytes fish is influenced by several factors such as the condition of nutrition, physical activity and age (Delman & Brown, 1989).

All treatments decreased the number of erythrocytes after challenge test with the bacteria *E. tarda*. The lowest number of erythrocytes occurs in treatment C around 0.530.01 %, while the highest found in treatment B. Based on the analysis of variance showed there was no significant difference between treatments after the challenge test. This means that the administration of lactic acid bacteria isolates did not give a significant effect on the number of carp erythrocytes after challenged tested. However, the largest decline occurred in treatment C of 48 ± 0.06 % and the lowest decline found in treatment A by 39 ± 0.21%.

Decreased erythrocytes after the challenge test showed phagocytosis of the bacterium *E. tarda*. As according to Matofani et al. (2013), in the phagocytosis, the process requires oxygen which results in a decreased the addition of erythrocyte, due to the extracellular product derived from *E. tarda* are capable

to lyse erythrocytes. A decrease in erythrocytes after a challenge test can also be caused by an injury resulting in blood coming out of the veins. Alsaid et al. (2015) stated that the damage to the body that occurs during infection can reduce the number of erythrocytes.

Table 2. The decrease percentage erythrocytes after immersion with LAB isolates and the Test Challenge with *E. tarda*

Treatment	The average of erythrocytes (10^6 cells/mm3)		The decrease persentage of erythrocytes (%)	Total erythrocytes (10^6 cell/mm^3)		The decrease persentage of of erythrocytes (%)
	Before immersion	After immersion		After immersion	Challenge Test (in vivo)	
Control (A)	1.05 ± 0.05	1.01 ± 0.02	-3% ± 0.047[a]	1.01 ± 0.02	0.62 ± 0.08	39 ± 0.21[a]
CcB7 (B)	1.05 ± 0.04	1.13 ± 0.03	7% ± 0.077[a]	1.13 ± 0.03	0.63 ± 0.02	44 ± 0.07[a]
CcB8 (C)	1.03 ± 0.04	1.03 ± 0.01	-1% ± 0.045[a]	1.03 ± 0.01	0.53 ± 0.01	48 ± 0.06[a]
CcB15 (D)	1.05 ± 0.03	1.03 ± 0.04	-2% ± 0.058[a]	1.03 ± 0.04	0.56 ± 0.02	45 ± 0.11[a]

Description: The same letter are not significantly different from each other (P>0.05 ANOVA)

Total of Hematocrit After Immersion with LAB Isolates and the Test Challenge with *E. tarda*

The immersion with LAB isolates (treatment B, C and D) increased hematocrit of the fish, while the control experienced a slight decline. The highest hematocrit levels found intreatment C (CcB8) by 22.76 ± 2.03 %, while the lowest found in treatment B (CcB7) around 19.65± 3.52% (Table 5). Table 5 shows the highest percentage increase in treatment B, as much as 21 ± 0.24% although treatment B had the smallest hematocrit levels. Treatment A decreased hematocrit levels of 3 ± 0.13% (Table 3). This shows LAB isolates as immunostimulant can improve the body health, as according Lukistyowati (2012) hematocrit value can be used as an indicator of the health of the fish after administration immunostimulant. Bastiawan et al. (2001) stated that the level of hematocrit value can describe the health condition of the fish. Low hematocrit indicates fish vitamin deficiency or anemia. While high hematocrit shows that fish is under stress. Hematocrit values may change depending on the temperature, feeding the fish healthy, and endurance (Bond, 1979).

Table 3. The increase percentage hematocrit after the immersion with LAB isolates and percentage of hematrocit leves after challenge with *E. tarda*

Treatment	Hematocrit levels after the immersion with LAB isolate		Average percentage (%)	Hematocrit levels after challenge with *E. tarda*		Average percentage (%)
	Before immersion	After immersion				
Control (A)	21.30 ± 4.42	20.85 ± 3.92	-3 ± 0.13[a]	20.85 ± 3.92	18.33 ± 5.77	-17.25 ± 0.23[a]
CcB7 (B)	15.14 ± 3.52	19.65 ± 3.52	21 ± 0.24[a]	19.65 ± 3.52	21.67 ± 2.89	7.31 ± 0.26[a]
CcB8 (C)	21.16 ± 2.61	22.76 ± 2.03	6 ± 0.19[a]	22.76 ± 2.03	20.88 ± 3.76	-11.06 ±0.19[a]
CcB15 (D)	19.39 ± 1.05	22.02 ± 2.64	11 ± 0.15[a]	22.02 ± 2.64	20:37 ± 3.21	-9.61 ± 0.18[a]

Description: The same letter are not significantly different from each other (P>0.05 ANOVA). The (-) sign indicates the decrease

Jurnal Biodjati 5(1):138-152, May 2020

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

After the challenge test hematocrit declines in all treatments except the treatment B. Analysis of variance results showed that after the challenge test there are no significant differences in hematocrit levels of fish among the treatments. This shows that the administration of lactic acid bacteria isolates did not affect hematocrit levels (Table 3). The decrease in hematocrit occurred due to a bacterial infection that causes by *E. tarda* leads to worsening fish health condition. During infection, fish showed a decline in feeding response so that the nutrients that enters the body decrease. As explained before, nutrition is one determinant of the hematocrit levels. Besides, the wounds caused by *E. tarda* infection can also cause a decrease in hematocrit (Alsaid et al., 2015). Yu et al. (2010) reported a decrease in hematocrit indicates anemia in fish.

Differential of Leukocytes After Challenge with *E. tarda*

The Percentage increase Monocytes, Neutrophils and Lymphocytes levels after challenge with *E. tarda* showed in Table 4. Based on result that the highest number of monocytes after challenge test was found in treatment B (CcB7) as much as 18%, the lowest was treatment A by 12%. While the increase in treatment C and D as much as 16% and 15%. The largest percentage increase in monocyte occurred in treatment B that is equal to 20 ± 0.053%. The increase in the percentage of monocytes was caused by a bacterial infection. The inflammatory process that occurs when the tissue damage caused by bacterial infection leads to the increasing of monocytes production. When infection occurs, the maturation of monocytes into macrophages happens quicker which leads to the tissue damage (Maftuch, 2007). According to Svobodová & Vykusová, (1991), the normal range of monocytes in the carp ranging between 3-5%. Moyle & Cech (1988) stated that the number of monocytes in the white blood cell population is little, but the number will increase if there is a foreign substance in tissue or circulation.

While the neutrophils carp fingerlings has increased after the challenge test. The highest increase in neutrophils counts was in treatment B as much as 11%. While in treatment A, C and D the increase occurred up to 9%, 9% and 10% respectively. The largest percentage increase in neutrophils occurred in treatments B and D respectively 62 and 63%. The increase in the number of neutrophils is part of the immune response of fish. Neutrophils in the blood act as the body's first defense and will increase if there is an infection (Harikrishnan et al., 2010). Suhermanto et al. (2011) stated that the primary function of neutrophils is a destroyer of foreign material through the process of the chemotactic phagocytic through cell adhesion, cell ingestion and destruction of the particle by lysosomal enzymes in phagolysosome.

The number of carp lymphocytes decreased in all treatment after the challenge test. The smallest decreased of carp lymphocytes occurred in the treatment B up to 71%. While in the treatment A, C and D showed decreased up to 78%, 75% and 75% respectively. The largest percentage of decreased lymphocytes occurred in treatment B namely - 9% (Table 9). Decreased lymphocyte occurs because of the foreign antigen into the body (Bijanti, 2005). Tizard (1982) reported a decrease resulting from the withdrawn of peripheral blood lymphocytes from the circulation into the inflamed tissue. The prolonged stress will increase cortisol levels in the blood, causing a loss of lymphocytes in the circulation and lymphoid organs. Wounds caused by infection can lead to a decreasing number of lymphocytes in the blood.

Table 4. The Percentage increase Monocytes, Neutrophils and Lymphocytes levels after challenge with *E. tarda*

Treatment	Monocytes			Neutrophils			Lymphocytes		
	After immersion	After the Test Challenge	Average percentage increase (%)	After immersion	After the Test Challenge	Average percentage increase (%)	After immersion	After the Test Challenge	Average percentage increase (%)
Kontrol (A)	11±1.0	12±0.0	9	6±2	9±1	37	83±1.15	78±0.58	-5
CcB7 (B)	15±1.0	18±1.0	20	7±1	11±2	62	78±1.73	71±1.15	-9
CcB8 (C)	14±1.0	16±0.6	12	8±1	9±1	22	78±1.53	75±0.00	-4
CcB15 (D)	14±1.0	15±0.6	5	6±2	10±1	63	80±1.53	75±1.00	-6

Body Damage of Carp After Infected *E. tarda*

The damage that occurs in fish after infected with *E. tarda* varies among fish. Treatment A showed clinical symptoms of body damage from the first day of observation, the color of fish scales changed on 2nd day and 3rd day. On 4th day, abdominal swelling began to appear. Body damage in the form of wound, pale scales and abdominal bloating occurred from 4th to 11th day. After that, the symptoms of body damage decreased.

Treatment B showed body damage since 2nd day and 3rd day. As in the control fish, first body damage appeared in the form of the appearance of lesions. Pale scales and discoloration began to appear on the 4th, 5th or 7th day of treatment. Treatment B showed no symptom of abdomen swelling. It is presumed that fish immune system in treatment B can lower the rate of infection of fish thus wound and skin color back to normal from 10th day. The symptom of body damage on fish di the reatment C starting on day 1 post infected. Full body damage began to occur on days 4 and 5 for at least two days. Furthermore, the fish continued to show recovery from 12th-14th day.

As with treatment A, B and C, the carp treatment D also suffered body injuries. In the treatment of D, lesions appear on 1st day and 2nd day. Then on the 4th day and 5th day discoloration of the scales begin to appear. Abdominal swelling also occurred on the 7th day and 8th day. The body damage started to undergo healing on the 13th day.

The damage that occurs in fish after infected by *E. tarda* varies each fish. *E. tarda* attack on the carp fingerlings caused hemorrhage (Figure 1a), pale scales, fin rot (Figure 1b), ulcer (Figure 1c) and dropsy (Figure 1d). The body damage caused by bacteria *E. tarda* on carp also showed clinical symptoms of wound redness (hemorrhage), fin rot (Sarjito et al., 2012). Then Prastiti et al. (2015) *E. tarda* on carp cause ulcers on the injection site, the body color of the fish being blackened, the appearance of lesions or ulcers, bleeding (hemorrhage), a bulging stomach (dropsy) and rot on the tail and fins.

Feeding Response of Carp After the Challenge Test with *E.tarda*

Response to the fish feed after infected with *E. tarda* varies between fish. Feeding response of the fish in the treatment B, C and D started to increase on 4th day after the test, while the days before it was hard to observe the response (no response). The normal feeding response started to appear on the 10th day - 11th day. While on treatment A, feeding response started to appear on days 5-6 and normal feeding response at 14th day (Table 5). These feeding response affect nutrients intake into the body of the fish that eventually

caused a decrease in red blood cell count and hematocrit carp after the challenge test. Prastiti et al. (2015) stated after infection *E. tarda* fish decreased appetite.

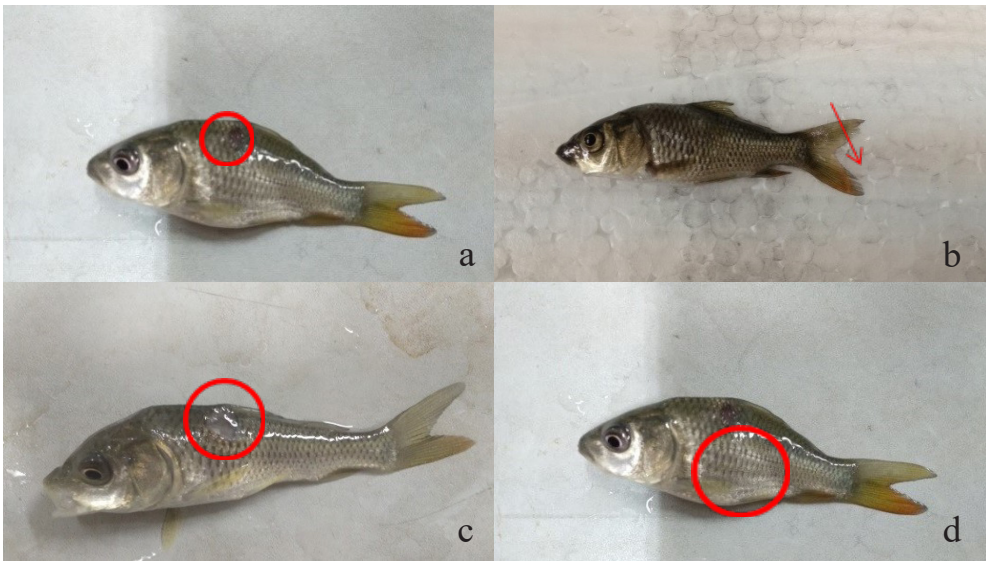

Figure 1. Body damage of carp fingerling infected by *E. tarda*; a. Hemorrhage; b. Fin rot; c. Ulcer; d. Dropsy

Table 5. Feeding response of carp after the challenge test with *E. tarda*

Treatment	Repeatation	Days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	-	-	-	+	+	+	+	+	+	+	+	+	+	++
	2	-	-	-	-	-	+	+	+	+	+	+	+	+	++
	3	-	-	-	-	-	+	+	+	+	+	+	+	+	++
B	1	-	-	-	+	+	+	+	+	+	++	++	++	++	++
	2	-	-	+	+	+	+	+	+	+	++	++	++	++	++
	3	-	-	-	+	+	+	+	+	+	++	++	++	++	++
C	1	-	-	+	+	+	+	+	+	+	+	++	++	++	++
	2	-	-	-	+	+	+	+	+	+	++	++	++	++	++
	3	-	-	-	+	+	+	+	+	+	+	+	++	++	++
D	1	-	-	-	+	+	+	+	+	+	+	++	++	++	++
	2	-	-	-	+	+	+	+	+	+	+	++	++	++	++
	3	-	-	-	-	+	+	+	+	+	+	++	++	++	++

(-) no response
(+) lower feeding response
(++) the normal feeding response

The Carp Response to Shock After the Challenge Test with *E. tarda*

The fish response to the shock was observed after the challenge test with the bacteria *E. tarda*. The observation was carried out tapping the wall of the aquarium. The response to shock on the fish seed after infection *E. tarda* varies each fish (Table 6). Treatment A or controls showed no response to a shock until 6th day. Then a low response on 7th day to 12th day began to appear. The normal response occurred on 13th day. This is allegedly due to the fish's body's defense back normal. While in treatment B, C and D a low response to shock started to appear since the 3rd day and 4th day. The normal shock response of fish in treatment B occurred from 7th day, while in the treatment C and D, it was strated on on the 8th day and 9th day respectively.

Table 6. The carp response to shock after the challenge test with *E. tarda*

Treatment	Repeatation	Days													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	1	-	-	-	-	-	-	+	+	+	+	+	+	++	++
	2	-	-	-	-	-	-	-	+	+	+	+	+	++	++
	3	-	-	-	-	-	-	+	+	+	+	+	+	++	++
B	1	-	-	+	+	+	+	++	++	++	++	++	++	++	++
	2	-	-	-	+	+	+	++	++	++	++	++	++	++	++
	3	-	-	+	+	+	+	++	++	++	++	++	++	++	++
C	1	-	-	-	+	+	+	+	++	++	++	++	++	++	++
	2	-	-	-	+	+	+	++	++	++	++	++	++	++	++
	3	-	-	-	+	+	+	+	++	++	++	++	++	++	++
D	1	-	-	-	+	+	+	+	++	++	++	++	++	++	++
	2	-	-	-	+	+	+	+	+	++	++	++	++	++	++
	3	-	-	-	+	+	+	+	+	++	++	++	++	++	++

(-) no response
(+) lower respon to shock
(++) normal response to shock

Survival Rate of Carp After Infected *E. Tarda*

The survival of carp after immersion treatment with LAB isolates fish was relatively higher than the untreated one (control). The survival rate of the fish in the treatment B (isolates CcB7), C (CcB8) and D (CcB15), were 80%, 72% and 70% respectively. While the lowest survival rate was in the treatment A (control) by 40% (Figure 2), meaning that immersion with LAB isolates can increase the survival rate. Results of analysis of variance (ANOVA) on the percentage of survival rate indicates there is difference between treatments. Treatment A was significantly different from treatment B, C and D. This means that the administration of lactic acid bacteria affects the survival rate of carp seed after infected bacteria *E. tarda*. While on treatment B, C and D show no difference means giving LAB isolates produce level survival was equally good.

The effectiveness of LAB in improving the body's resistance was also evident in the research of Pimentel & Katagiri (2008) that showed the distribution of four species of *Lactobacillus* in tilapia gives different effects after challenge with bacteria tested *E. tarda*, char-

acterized by increased responsiveness of macrophages and lower fish mortality. According to Andayani et al. (2017), the provision of the LAB species *Lactobacillus plantarum* effect on the survival of catfish (*Pangasius djambal*) that were infected with *E. tarda* up to 90%.

Judging from the value of survival, the isolates CcB7 (B) is capable of producing the highest survival. This is presumably because the bacterial isolates CcB7 can produce higher secondary metabolites to release a variety of chemicals that can stimulate and induce the proliferation of immune cells, such as T and B lymphocytes, and macrophages. Moreover, isolates CcB7 is allegedly able to adapt well in the body of tested fish and increase better endurance infish. This is also the cause of isolates CcB7 able to reduce body damage caused by the bacterium *E. tarda* leads to fewer fish mortality.

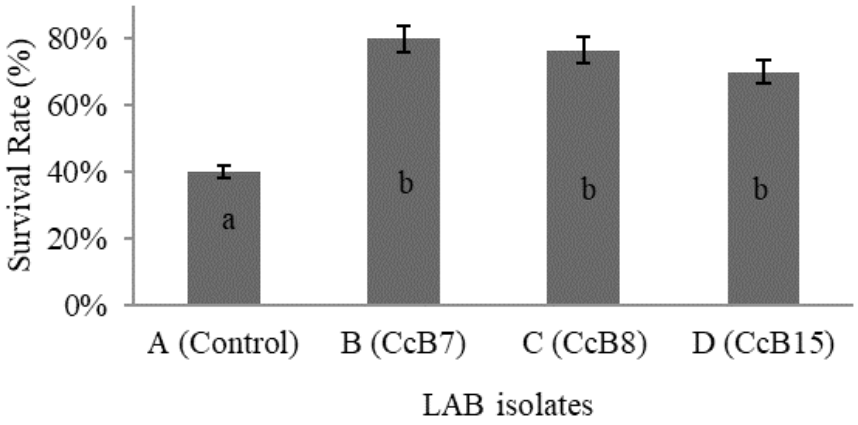

Figure 2. Survival rate of common carp after infected *E. tarda*

Based on the results of this study, it can be concluded that all LAB isolates used in this study can improve the body's resistance carp seed. LAB isolates CcB7 is most effective for increasing the endurance and survival rate of carp fingerlings up to by 80%. he LAB isolates increased the levels of leukocytes, erythrocytes and hematocrit the highest by 18 ± 0.057%, 7 ± 0.077% and 0.26 ± 7.31% respectively. The challenge test with the bacteria *E. tarda* produced mild clinical symptoms, an increase in monocyte and neutrophil by 20% and 62%, and caused a decline in the number of lymphocytes up to - 9 %.

ACKNOWLEDGEMENTS

Further thanks to the Faculty of Fisheries and Marine Sciences who have given all the trimmings and to all those who have been helped this research.

REFERENCES

Allameh, S. K., Noaman, V. & Nahavandi R. (2017). Effects of Probiotic Bacteria on Fish Performance. *Advanced Techniques in Clinical Microbiology*, 1(2), 1-5.

Alsaid, M., Abucelliana, A. F., David, H. H., Mustapha, N. M., Bejo, S. K., Abdelhadi, H. E. & Ramdan, R. H. (2015). Haematological, Biochemical Changes and Clinical Signs Following Experimental Infection of *Streptococcus agalactiae* in Red Hybrid Tilapia (*Oreochromis* sp.). *Basic Research Journal of Agricultural Science and Rievew*, 9(9), 289-295.

Amezquita, A. & Brashears, M. M. (2002). Competitive Inhibition of *Listeria monocytogenes* in Ready to Eat Meat Products by Lactic Acid Bacteria. *Journal Food Protection*, 65(2), 316-325.

Anderson, D. P. & Siwicki, A. K. (1993). Basic Hematology and Serology for Fish Health Program. *Paper of Second Symposium on Aquaculture "Aquatic Animal Health and the Environment" Phuket*. Thailand.

Andayani, S., Suprastyani, H., Gumala, G. D. A., Octave, U., Fatikah, N. M., Wahyudi, M., Farina, A. & Primary, R. (2017). Effect of Bacterium *Lactobacillus plantarum* Against Histopathology and Hematology Jambal catfish (*Pangasius djambal*) Infected Bacteria *Edwarsiella tarda*. *FMR-Journal of Fisheries and Marine Research*, 1(1), 31-38.

Bastiawan, D. A., Wahid, Alifudin, M. & Agustiawan, I. (2001). Blood Picture Dumbo Catfish (*Clarias gariepinus*) were Infected by the Fungus *Aphanomyces* spp. At pH Layout. *J. Pen. Per. Indonesia*, 7(3), 44-61.

Bintoro, A. J. (2002). Probiotic Potential of *Lactobacillus acidophilus* and Microflora as Kefir as Immunostimulants. *Unpublished Thesis (BSc)*. Bogor: Department of Animal Production, Faculty of Animal Science, Institut Pertanian Bogor.

Bijanti, R. (2005). *Fish Hematology Blood Collection and Examination Techniques Fish Hematology*. Surabaya: Department of Basic Veterinary Medicine. Faculty of Veterinary Medicine. Universitas Airlangga.

Bond, C. E. (1979). *Biology of Fishes*. Philadelpia: Sounders College Publishing.

Brown, Esther, M. & Dellman, H. D. (1989). *Textbook of Veterinary Histology, third edition*. Jakarta: The publisher University of Indonesia.

Department of Fisheries and Marine. (2018). *Annual Report 2015/2016*. Jawa Barat.

Efendi, H., Agusnimar & Warman, E. (2016). Effect of Soaking Time Span Differences In Solution Probiotik larvae survival and Growth of Fish Slice (*Kryptopterus lais*). *Journal of Agricultural Dynamics*, 32, 143-150.

Effendie, M. I. (1997). *Fisheries Biology*. Yogyakarta: Nusatama Library Foundation.

Gaspersz, V. (1991). *Experimental Design Method*. Bandung: CV. ARMICO.

G'omez, G. D. & Balc'azar, J. L. (2008). A Review of the Interactions Between Gut Microbiota and Innate Immunity of Fish. *FEMSImmunology and Medical Microbiology*, 52(2), 145-154.

Harikrishnan, R., Balasundaram, C., Kim, M. C., Kim, J. S., Han, Y. J. & Heo, M. S. (2009). Innate Immune Response and Disease Resistance in *Carassius auratus* by Triherbal Solvent Extracts. *Fish and Shellfish Immunology*, 27(3), 508-515.

Klontz, G. W (1994). *Techniques in Fish Immunology*. USA: Depertement of Fish and Wild life resource. University of Idaho.

Lukistyowati, I. (2012). Effectiveness studies Bitter (*Andrographis paniculata* Nees) to Prevent Edwardsiellosis In Catfish (*Pangasius hypopthalmus*). *Periodical Journal of Terubuk Fisheries*, 40(2), 56-74.

Maftuch. (2007). Exposure to *Vibrio alginolyticus* Grouper Against Intestinal Histopathology Rats (*Cromileptes altivelis*) and Increasing Number and Macrophage Cells Activity. *Fisheries Research Journal*, 10(1), 66-70.

Matofani, A, Hastuti, S. & Basuki, F. (2013).

Blood Profiles Kunti Tilapia (*Oreochromis niloticus*) yang Diinjeksi *Streptococcus agalactiae* With Berbeda. *Journal density of Aquaculture Management and Technology*, 2(2), 64-72.

Mohanty, B. R. & Sahoo, P. K. (2008). Edwardsiellosis in fish: A brief review. *Journal of Biosciences*, 32(7), 1331-1344.

Moyle, P. B. & Cech, J. J. (1988). *Fishes: an Introduction to Ichtyology*. USA: Prentice Hall, Inc.

Nurjannah, R. D. D., Prayitno, S. B., Sarjito. & Lusiash, A. M. (2013). Effect of Leaf Extract Soursop (*Annona muricata*) Against Profile Blood and the survival of Goldfish (*Cyprinus carpio*) infected Bacteria *Aeromonas hydrophila*. *Journal of Aquaculture Management and Technology*, 2(4), 72-83.

Pangaribuan, D. R., Tumbol, R. A., Manopo, H. & Sampelako, J. (2013). Bekasang Role as an Immunostimulant to the Non-Specific Immune Response in Tilapia (*Oreochromis niloticus*). *Journals. Aquatic Science & Management*, 1(2), 165-170.

Pimentel, S. S. & Katagiri, T. (2008). Differences of Probiotic Effects on *Edwardsiella tarda* Challenged Nile Tilapia (*Oreochromis niloticus*) Fed with Four *Lactobacillus* Species. *Sci aquaculture*, 56(3), 401-408.

Pinoke, A. J., Steven, R. A., Tumbol, M. E. & Kolopita, F. (2015). The Addition of Feed Seed Bakasang on Eels (*Anguilla marmorata*) to Enhance Non-Specific Immune System. *Journal of Aquaculture,* 3(3), 12-18.

Prastiti, L. A., Sarjito & Slamet, B. P. (2015). Addition Effect of Red Ginger Extract (*Zingiber officinale* var. *Rubrum*) on Maintenance Media Against Survival and Growth Gurami (*Osphronemus gouramy*) Infected Bacteria *Edwardsiella tarda*. *Journal of Aquaculture Management and Technology*, 4(3), 31-37.

Rahmawati, A., Union, P. & Kurniasih. (2013). Histopathologic with *Edwardsiella tarda* as Cause of Death Goldfish Koki (*Crassius auratus*); Koch's postulates. *Journal of the San Veterner*, 31(1), 55-65.

Rao, P., Srinivisa, S., Meng, L. T. & Yin, L. K. (2001). *Oposonized Edwardsiella tarda Virulent Strains are Able to adhere to and Survive and replicate within Fish Phagocytes but Fail to Stimulate Reactive Oxygen Intermediates*. USA: American Society For Microbiology.

Roberts, R. J. (2001). *Fish Pathology*. London: Bailliere Tindal.

Sarjito, Minaka, A., Rajasa, O. K., Sabdono, A. & Prayitno S. B. (2012). The Richness of Bacteria Associated with Bacterial Diseases on the Giant Gouramy (*Osphronemos gouramy*). *Procceding International Conference of Aquaculture Indonesia (ICAI)*. Published Indonesian Aquaculture Society Publisher Agency Semarang.

Septriani, E. H. & Wardiyanto. (2012). Effect of Different Time Giving probiotics Non-Specific Immune Response Against Goldfish (*Cyprinus Carpio* L.) The Tested Challenge with bacterium *Aeromonas salmonicida*. *E-Journal of Aquaculture Engineering and Technology*, 1(1), 39-46.

Suhermanto, A., Andayani, S. & Mafuch. (2011). The Provision of Total Phenol Sand Sea Cucumber (*Holothuria scabra*) to Improve Differential Leukocyte and Goldfish (*Cyprinus carpio*) Infected Bacteria *Aeromonas hydrophila*.

Journal of Marine, 4(2), 150-157.

Sukenda, Jamal, L., Wahyuningrum, D. & Hasan, A. (2008). The Use of Chitosan for Infection Prevention *Aeromonas hydrophila* on Dumbo Catfish *Clarias* sp. *Indonesian Aquaculture Aquaculture Journal*, 7(2), 159-169.

Svobodová, Z. & Vykusová, B. (1991). *Diagnostic, Prevention and Therapy of Fish Disease and Intoxication*. Czechoslovakia: Research Institute of Fish Culture and Hydrobiology Vodňany.

Tizard, I. R. (1982). *An Introduction of Veterinary Immunology*. Philadelphia, USA: WB Saunders Company.

Vasiljevic, T. & Shah, N. P (2008). Probiotics-from Metchnikoff to Bioactives. *International Dairy Journal*, 18(7), 714-728.

Yu, J. H, Han, J. J. & Park, S. W. (2010). Haematological and Biochemical Alterations in Korean Catfish *Silurus asotus*, Experimentally Infected with *Edwardsiella tarda*. *Aquaculture Research*, 41(2), 295-302.